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(54) Title: FEED ADDITIVE FOR POULTRY, POULTRY FEED AND METHOD OF POULTRY FEEDING

#### (57) Abstract

The feed additive for poultry comprises a cellulase or a xylanase producible by means of selected strains of Humicola insolens and Trichoderma longibrachiatum. By means of this additive the malabsorption syndrome is effectively alleviated.

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# FEED ADDITIVE FOR POULTRY, POULTRY FEED AND METHOD OF POULTRY FEEDING

The invention comprises a feed additive for poultry, a poultry feed, a method of poultry feeding, and a use of the feed additive for poultry. The feed additive is used as a medicament for alleviation of the malabsorption syndrome in broiler chickens.

Malabsorption syndrome is a problem observed in broilers in many countries of the world. This syndrome causes very important losses to the poultry industry. The affected flocks show several variable clinical symptoms:

- Lower digestion of feeds causing diarrhoea, particularly fats and fat soluble substances, or only wet litter,
  - reduced fat digestability
  - poor growth (runting or stunting syndrome)
  - high feed:gain ratio
- 15 brittle bones

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- defective feathering
- defective pigmentation of the skin (pale bird syndrome): The yellow colour of affected birds is less than normal and the pigmentation is quite variable between birds of a same flock; this defective pigmentation is due to a bad utilization of the pigments called xanthophylls contained in feeds.

Sometimes only one of these symptoms exists in a flock. Sometimes several symptoms can be seen in the same flock.

The etiology of the syndrome is not well determined.

Several viruses have been cited as possible causal agents of the syndrome, e.g. Rotavirus, reovirus, coronavirus, adenovirus, calicivirus, parvovirus, and togavirus.

Mycotoxins have also been cited as possible causal agents of the syndrome.

The invention describes a feed additive for alleviation of the malabsorption syndrome in poultry, a poultry feed, and a method of poultry 5 feeding.

The feed additive for alleviation of the malabsorption syndrome in poultry according to the invention is characterized by the fact that it comprises a cellulase and a xylanase producible by means of *Humicola insolens* (DSM 1800) or *Trichoderma longibrachiatum* (NRRL 11460). NRRL 11460 was originally deposited as *Trichoderma reesei* and later reclassified as *Trichoderma longibrachiatum*. Surprisingly it has been found that this feed additive containing as the active component a specific cellulase/xylanase combination exhibits the ability effectively to alleviate the malabsorption syndrome in poultry.

Due to the fact that part of the malabsorption syndrome comprises a reduced fat digestibility, the obvious constituent of a feed additive for alleviation of the malabsorption syndrome would be a lipase. According to the invention, however, it surprisingly has been found that lipase does not alleviate the malabsorption syndrome to any significant degree. Even more surprisingly, as above indicated, it has been found, that the above indicated feed additive with the most specific cellulase/xylanase combination effectively alleviates the malabsorption syndrome, despite the fact that no corresponding theoretical explanation at present can be provided.

It belongs to the prior art that the growth of poultry can be improved by addition of cellulase and/or xylanase to the feed, vide e.g. SU patent no. 933.063 (referenced in Derwents abstract in World Patent Index Latest), and DK patent application no. 805/88, and selected cellulase/xylanase enzyme complexes for that purpose are described in SU patent no. 577.230 (referenced in Derwents abstract in World Patent Index), and DE 1.904.239. However, the feed:gain ratio of the prior art feeds are not satisfactory, and it 30 has been found that the feed:gain ratio of the poultry feed according to the

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invention is significantly better than the feed:gain ratio of the prior art poultry feeds, vide Example 6. More important however, according to the invention we are able to offer an alleviation of the symptoms of the malabsorption syndrome which is not possible according to the prior art. There is also the advantage that due to this improvement less manure has to be disposed of according to the invention, whereby less pollution is generated according to the invention.

The poultry feed according to the invention is characterized by the fact that the poultry feed is composed of a normal poultry feed, to which a feed additive according to the invention has been added.

The method of poultry feeding according to the invention is characterized by the fact that the poultry feed is the poultry feed according to the invention. Thus, the method according to the invention consists in administrating specific cellulase/xylanase enzyme combinations, by oral route, to birds raised in areas where the malabsorption syndrome exists endemicly.

A preferred embodiment of the method according to the invention is characterized by the fact that the feed additive according to the invention is added to the drinking water for the poultry.

Another preferred embodiment of the method according to the invention is characterized by the fact that the feed additive according to the 20 invention as a solid material is added to the normal poultry feed.

the growth of chickens when such enzymes are incorporated into feeds and the range of enzymes includes amylase, cellulase, hemicellulase, glucanase, lipase, gumase, chitinase and cytolytic enzymes. However, the specific combination of the enzymes producible by means of *Humicola insolens* (DSM 1800) or *Trichoderma longibrachiatum* (NRRL 11460) is needed.

The present invention demonstrates how the addition of a selected cellulase/xylanase enzyme complex is able to alleviate the effects of malabsorption syndrome and return the performance of the chickens to their

normal state. It is also demonstrated that traditional cellulases do not have the same effect.

The examples given below demonstrate the properties and advantages of the invention.

SP 343 is the *Humicola insolens* cellulase/xylanase complex from DSM 1800, and SP 431 is the xylanase from *Trichoderma longibrachiatum* (NRRL 11460).

#### **EXAMPLE 1**

160 chicken were taken randomizedly, at 8 days of age, from a 10 flock raised in an area where malabsorption syndrome was endemic.

These 160 chicken were afterwards raised in individual cages, on wired floor. Each cage was equipped with a feeder and a waterer in order to provide feed and water ad libitum.

The 160 chicken were divided in 4 lots of 40 birds. Each lot re-15 ceived one of these 4 experimental feeds:

- 1) Basal feed wheat based (control)
- 2) Basal feed supplemented with enzyme SP 343 at 200 g/ton
- 3) Basal feed supplemented with enzyme SP 343 at 1000 g/ton
- 4) Basal feed supplemented with Lipozyme 10,000 L at 50 g/ton

After 8 days of experiment, feces were collected for each bird for 3 days. The collected feces of 2 birds consuming the same feed were pooled together: Results of the nutritional balance trial were obtained with 20 individual data for each feed.

Dry matter and fat were analysed in feeds and feces in order to determine dry matter and fat digestibility according to the well known procedure commonly used in animal nutrition.

The results were submitted to variance analysis and Duncan's comparison of the means.

The results obtained (table 1) show that the SP 343 enzyme improved dry matter and fat digestibility but that the lipase (Lipozyme 10000 L) was not able to improve digestibility of these components.

Table 1

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	Lots	Feed	Dry matter Digestibility (%)	Fat Digestibility (%)
15	1	Basal	63.0±4.2 A	43.1±17.1 A
	2	Basal +Enzyme 200 g/t	67.8±3.9 B	54.8±14.7 B
20	3	Basal +Enzyme 1000 g/t	68.7±3.4 B	58.0±11.5 B
25	4	Basal +Lipase	59.7±5.3 A	36.6±17.4 A
25	Variance ar	nalysis	p≤0.001	p≤0.001

(In a same column, A and B means a significant difference (p $\le$ 0.001) with Duncan's test).

#### **EXAMPLE 2**

320 chicken were taken, at 8 days of age, from a flock raised in an area where malabsorption syndrome was endemic.

The birds were divided in 4 lots of 80 birds.

The birds were raised in individual cages as previously described in Example 1.

Each lot of birds received one of these 4 experimental feeds, for three weeks (from 8 to 29 days of age):

- 1) Basal feed
- 10 2) Basal feed supplemented with enzyme SP 343 at 200 g/ton
  - 3) Basal feed supplemented with enzyme SP 343 at 1000 g/ton
  - 4) Basal feed supplemented with Lipozyme 10000 L at 50 g/ton

Body weight gain, feed intake and feed:gain ratio were measured for each bird from 8 to 29 days of age.

Results were submitted to statistical analysis as previously described.

The results (table 2) show that birds consuming the basal feed had high feed:gain ratio. The SP 343 enzyme improved growth, and decreased feed intake and feed:gain ratio significantly. The lipase enzyme added at a dose rate in excess of the lipase side activity present in SP 343 did not significantly change the feed:gain ratio.

Table 2

5	Lots	Feeds	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed intake (g)	Feed:gain ratio
	1	Basal	142.4	925.8a (100.0)	783.4a (100.0)	1487.0a (100.0)	1.904A (100.0)
<b>10</b>	2	Basal +Enzyme 200 g/t	142.9	984.8ab (102.6)	806.9ab (103.0)	1479.8ab (99.5)	1.842B (96.7)
	3	Basal +Enzyme 1000 g/t	143.0	960.7b (103.8)	817.7b (104.4)	1439.3b (96.8)	1.767C (92.8)
15	4	Basal +Lipase 50 g/ton	142.4	940.6ab (101.6)	798.2ab (101.9)	1504b (101.2)	1.892A (99.4)
20		ance ysis	p≤0.05	p≤0.05	p <b>≤0.05</b>	p≤0. <b>05</b>	p≤0.001

In a same column, Duncan's test:

a, b

p≤0.05

A, B, C

p≤0.001

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#### **EXAMPLE 3**

320 broilers were taken, at 20 days of age, from a farm located in an area where malabsorption syndrome was endemic.

After being weighed, they were distributed in 4 lots of 4 repli-5 cations of 20 birds for each replication.

The birds were raised on floor pen with wood shaving litter (2 m<sub>2</sub>/pen).

Each lot of birds received one of these 4 experimental feeds, from 20 to 41 days of age (21 days of experiment):

- 10 1) Basal feed 1 maize base
  - 2) Basal feed 1 supplemented with enzyme SP 343 at 1000 g/ton
  - 3) Basal feed 2 wheat base
  - 4) Basal feed 2 supplemented with enzyme SP 343 at 1000

15 g/ton

The xanthophyll composition of the 2 basal feeds are on table 3.

Table 3

		Maize base	Wheat base
20	Lutein (ppm)	41.80	42.90
	Zeaxanthin (ppm)	13.00	12.00
	Canthaxanthin (ppm)	0.92	1.03

On the 14th day of the experiment, blood plasma was collected from 12 birds of each treatment (3 birds of each replication) for measuring the

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optical density by spectrophotometry (with a spectrophotometer Beckman DU 64) at 450 nm. The optical density of plasma reflects the absorbed xanthophyll content of the blood.

Weight gain, feed intake and feed:gain ratio of the birds were also measured for the 21 days of experiment.

After the 21 days of experiment all the birds were slaughtered in an industrial abattoir. After cooling for 24 hours, the pigmentation of the carcasses was ranked by 2 well trained quality controllers of the abattoir. Each bird was given a score:

1: badly pigmented2: moderately pigmented3: well pigmented

The average score was calculated for each treatment.

RESULTS
Optical Density of Plasma

Table 4

20	Lots	Feeds	Optical Density	Coefficient of Variation (%)
	1	Maize base	0.441 ± 0.173	39.3
٠	2	Maize base + enzyme	0.605 ± 0.163	27.3
	3	Wheat base	$0.407 \pm 0.173$	43.7
25	Wheat base + enzyme		0.615 ± 0.117	19.0
	Variance	analysis	p≤0.001	

Table 4 shows that optical density was significantly increased by the enzyme. There were therefore more xanthophylls in the blood.

Coefficient of variation of optical density was also lower with birds receiving enzyme: The xanthophyll contents of the blood were more 5 homogenous with enzyme supplemented feeds than with control feeds.

#### Pigmentation of Carcasses

Table 5 shows that the pigmentation score of carcasses was increased by the enzyme. In basal feeds, 2 to 5 percents of birds were scored as badly pigmented, while there was no such scored bird with enzyme.

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Table 5

	Lots	Feeds	Score	Contr.1 %birds	Score	Contr.2 %birds	Score	Average Score
5			3	20		20		
	1	Maize base	2	.77 3	2.17	78 2	2.18	2.175
		Maize base	3	31		25		
20	2	+ Enzyme	2 1	69 0	2.31	75 0	2.25	2.280
			3	14		15		
25	3	Wheat base	<b>2</b> 1	84 2	2.12	<b>80</b> <b>5</b>	2.10	2.110
		Wheat base	3	29		30		
30	4	+ Enzyme	2 1	71 0	2.29	70 0	2.30	2.295

#### Zootechnical Performances

			Table 6		
	Lots	1 .	2	3	4
5	Feeds	Maize base	Maize base + enzyme	Wheat base	Wheat base + enzyme
	Live weight (g)				•
	initial	510±5	512±8	520±1	1517±15
10	final	1651±4	1661±28	1656±39	1632±71
	Weight gain(g)	1141±8	1150±25	1136±32	1114±57
•	Feed intake(g)	2570±79	2551±10	2608±123	2438±135
	Feed:gain ratio	2.253±0.078	2.219±0.052	2.296±0.072	2.188±0.039

Table 6 shows that the enzyme:

- Improved slightly growth of birds with maize based feed.
- Decreased feed intake and feed:gain ratio for both kinds of feeds.

#### **EXAMPLE 4**

#### 20 Digestibility Trials

Cecectomized cockerels are divided at random into groups of 6 birds. A basal wheat based feed is used supplemented with enzyme at the rate of 1 kg per ton. The birds are starved for 30 hours and then each fed 80 g of feed. Faeces are collected from each bird and then pooled for each treatment

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group. The pooled samples are oven dried at 70°C and then analysed by the current EEC methods except for fat which is analysed by acid hydrolysis.

The results in table 7a and 7b show that the specific cellulase/xylanase combination found in SP 343 and SP 431 are able to improve fat digestibility in the cecectomized cockerel but that lipase or standard cellulase from Tricoderma sp is not able to improve digestibility.

Table 7a

Enzyme	True Digesti	oility, %	
10	Dry matter	Fat	
Control	72.95	87.78	
SP 343	72.87	90.98	
SP 431	74.04	91.59	
15 Lipolase	71.62	87.98	

Table 7b

20	Enzyme	True Digestibility, %		
	Z. 12 / 110	Dry matter	Fat	
	Control	72.28	87.16	
	SP 343	72.26	88.52	
	Celluclast	72.36	86.73	
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#### **EXAMPLE 5**

Comparison of the Effect of a Conventional Cellulase and Growth Promoting

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468 days old chickens were placed into 18 pens each containing 5 26 birds (13 male and 13 female). The pens were randomly divided into 3 groups which received the basal feed (Table 8) with the following supplements:

Group A:

No supplement

Group B:

Celluciast 1.5 L at 1000 g/ton feed

Group C:

Virginianycin at 90 ppm

Feed and water were supplied ad lib and body weight gain, feed intake and feed conversion were measured over the 47 day period of the trial. The results were submitted to statistical analysis as previously described.

The results show (Table 9) that addition of a traditional cellulase preparation from Trichoderma reesei (Celluclast 1.5 L) did not significantly influence the feed intake or weight gain of broiler chickens fed a wheat based diet.

Table 8
Feed Composition for Example 5

		0-28 days	29-49 days
5	Wheat	58.0%	71.0%
	Soy bean meal	28.5%	15.0%
	Meat & bone meal	5.0%	5.0%
	Corn gluten	3.0%	3.0%
	Fat	2.0%	2.0%
10	Trace minerals/vitamins	3.5%	4.0%
	Crude Protein	23.8%	19.0%
	Energy (Kcal/kg)	2892	2992

Table 9
Feeding Results

15		recuirg results			
•		Α	В	С	
	Overall weight gain (kg)	2.09	2.11	2.18 <sup>a</sup>	
20	Overall feed intake (kg)	4.25	4.35	4.43 <sup>a</sup>	
	FCR	2.05	2.02	2.03	
				<u> </u>	

Values with a superscript a) in a single row are significantly different from the control (p $\le$ 0.05)

#### **EXAMPLE 6**

Comparison of the effect of a traditional cellulase (SP 346, commercial cellulase from *Trichoderma reesei*) with SP 343 (*Humicola insolens* cellulase/xylanase, DSM 1800), and SP 431 (*Trichoderma longibrachiatum* xylanase, NRRL 11460)

350 chickens were taken from a commercial flock, at 8 days of age and divided into 7 lots of 50 birds.

The birds were raised in individual cages as previously described in Example 1.

The birds were fed on a basal wheat based feed (Table 10) 10 supplemented with enzymes according to the following scheme:

SP 346	0.02% and 0.1%
SP 343	0.02% and 0.1%
SP 431	0.01% and 0.025%

Table 10

15	COMPONENT	% IN THE DIET
	Wheat	61.40
	Soybean meal 46% C.P.	22.00
	Fishmeal 65% C.P.	5.00
20	Meat and Bone meal 50% C.P.	3.30
	Calcium carbonate	0.61
	Sait	0.28
	DL-Methionine 98%	0.17
	L-lysine 78%	0.04
25	Animal fat	6.20
	Vitamin/Trace Mineral premix*	0.50
•	Chromium oxide	0.50

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	A.M.E. (Kcal/kg)	3,120
	Protein (%)	22.15
	Sulphur amino acids (%)	0.92
5	Lysine (%)	1.20
	Fat (%)	7.85
	Crude fiber (%)	3.00
	Calcium (%)	0.95
	Available Phosphorous (%)	0.40
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\* Supplies: monensin 100 ppm, bacitracin 50 ppm

Body weight gain, feed intake and feed:gain ratio were measured for each bird from 8 to 22 days of age. A balance experiment was also carried out in which the apparent digestibility of Dry Matter, Fat and Crude Fiber were 15 determined.

The results (Table 11) show that the performance of those chickens receiving the conventional cellulase (SP 346) was not significantly different to that of the control whereas those birds reciving SP 343 or the higher dose of SP 431 had a significantly improved Feed:Gain ratio.

Table 11

TREATMENTS

5		Control	0.02% SP 343	0.1% SP 343	0.02% SP 346	0.1% SP 346	0.01% SP 431	0.025% SP 431
	Weight gain (g	) 519.88	525.20	514.87	523.59	508.48	520.79	528.19
	(S.D.)	36.72	42.81	38.85	49.06	43.61	38.06	44.23
	Feed intake (g	775.38	765.86	752.59	773.15	754.10	763.94	765.58
10	(S.D.)	59.60	58.99	65.54	67.31	61.72	59.26	59.56
•	Feed:Gain ratio	1.493	1.460	1.462	1.479	1.485	1.468	1.452
	(S.D.)	0.070	0.066	0.063	0.070	0.068	0.063	0.077
	(0.2.)	(100)	(97.8)	(97.9)	(99.1)	(99.5)	(98.3)	(97.2)
		a	bc	bc	abc	ab	abc	C

15 Values for Feed:Gain ratio with a different letter are significantly different at a probability value p<0.5.

The results in Table 12 show that again only the SP 343 and SP 431 enzymes had a positive effect on the apparent digestibility of Dry Matter, Fat and Crude Fiber.

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	Apparent							
5	Digestibility (%)	Control	0.02% SP 343	0.1% SP 343	0.02% SP 346	0.1% SP 346	0.01% SP 431	0.025% SP 431
	Dry matter	73.31	76.16	75.31	74.20	72.64	75.12	74.07
	(S.D.)	1.75	1.41	1.57	0.70	1.31	1.23	1.29
		CD	A	AB	ВС	D	AB	ВС
	Fat	82.18	84.19	85.05	83.28	81.34	84.32	85.08
10	(S.D.)	59.60	58.99	65.54	67.31	61.72	59.26	59.56
		bc	ab	а	abc	C	ab	а
	Crude Fiber	6.13	14.22	9.28	5.56	4.04	11.49	6.48
	(S.D.)	5.64	4.82	4.52	3.10	4.56	6.10	7.87
		С	Α	ABC	С	C	AB	BC

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Values for Apparent Dry Matter or Crude Fiber Digestibility with a different letter are significantly different at a probability value p<0.001.

Values for Apparent fat Digestibility with a different letter are significantly different at a probability value p<0.05.

#### CLAIMS

- 1. Feed additive for alleviation of the malabsorption syndrome in poultry, characterized by the fact that it comprises a cellulase and a xylanase producible by means of *Humicola insolens* (DSM 1800) or *Trichoderma* 5 *longibrachiatum* (NRRL 11460).
  - 2. Poultry feed, characterized by the fact that the poultry feed is composed of a normal poultry feed, to which a feed additive according to Claim 1 has been added.
- 3. Method of poultry feeding characterized by the fact that the poultry is the poultry feed according to Claim 2.
  - Method according to Claim 3, characterized by the fact that the feed additive according to Claim 1 is added to the drinking water for the poultry.
- 5. Method according to Claim 3, characterized by the fact that the feed additive according to Claim 1 as a solid material is added to the normal poultry feed.

#### INTERNATIONAL SEARCH REPORT

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International Application No PCT/DK 90/00256

4 8, 1988 W.D. COWAN: "SELECTION AND APPLICATION OF ENZYMES TO IMPROVE UTILIZATION OF ANIMAL FEEDS", see page 1773 - page 1783  U.S., A., 3462275 (W.D. BELLAMY) 19 August 1969, see column 1, line 19 - line 21; column 4, line 67  Derwent's abstract, No. 78-649 21a/36, SU 577 230, publ. week 7836 MOSC FOOD IND TECHN; BIOTECH RES INST  EP, A2, 0280226 (SUOMEN SOKERI OY) 31 August 1988, see page 5, line 4 - line 46; claim 5  ** Special categories of cited documents: 10		ON OF SUBJECT MATTER (if several classification symbols apply, indic	
III. FIELDS SEARCHED  Minimum Documentation Searched*  Classification System  Classification System  Classification System  Documentation Searched other than Minimum Documentation to the Existent that such Documents are Individed in Preids Searched*  SE, DK, FI, NO classes as above  III. DOCUMENTS CONSIDERED TO BE RELEVANT*  Category*  Clastion of Document** with Indication, where appropriate, of the relevant passages**  Restorant to Claim No.**  Med. Fac. Landbouws. Rijksuniv. Gent., Vol. 53, No.  4a, 1988 M.D. COMAN: "SELECTION AND APPLICATION OF ANIMAL FEEDS**, see page 1773 - page 1783  X US, A, 3462275 (W.D. BELLAMY) 19 August 1969,  see column 1, line 19 - line 21;  column 4, line 67  X Derwent* sastract, No. 78-649 21A/36, SU 577 230,  publ. week 7836  MOSC FOOD IND TECHN; BIOTECH RES INST  EP, A2, 0280226 (SUOMEN SOKERI OY)  31 August 1988, see page 5, line 4 -  line 46; claim 5  ** Special categories of cited documents: 12*  ** Compand self-ping the general state of the art which is not considered to be of particular relevance in the published of the state the Informational Claims of the Propriet Search Search Search Search Report  ** Special categories of cited documents: 12*  ** Special categories of cited documents: 13*  ** Special categories of cited documents: 14*  ** Special categories of cited documents: 15*  ** Special categories of cited documents:			PC
Classification System  Classification System  Classification System  Classification System  Classification Symbols  Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched*  SE,DK,FI,NO classes as above  III. Documents Considered to BE Relevant*  Category*  Citation of Document,II with Indication, where appropriate, of the relevant passages 12  As 1988 ND. COMAN: "SELECTION AND APPLICATION OF ENZYMES TO IMPROVE UTILIZATION OF ANIMAL FEEDS*, see page 1773 - page 1783  X US, A, 3462275 (W.D. BELLAMY) 19 August 1969, see column 1, line 19 - line 21; column 4, line 67  X Derwent's abstract, No. 78-649 21A/36, SU 577 230, publ. week 7836  MUSC FOOD IND TECHN; BIOTECH RES INST  X EP, A2, 0280226 (SUOMEN SOKERI OY)  31 August 1988, see page 5, line 4 -  11-2  * Special categories of clied documents: 19  * Special categories of clied documents: 20  * Special categories of clied documents: 21  *	11PC5: A 23 N		
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	SERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE	
This intern	tional search report has not been established in respect of certain claims under Article 17(2) (a)	for the following reasons:
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	additional search fees were accompanied by applicant's protest.	
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### ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/DK 90/00256

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 90-11-28 The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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